

Applicant : Wei-Yu Lo et al.
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Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. (Previously presented) A Lac shuttle vector, comprising:
 - (a) a region which regulates a plasmid copy number, wherein said region comprises an E. coli replication origin sequence;
 - (b) a eukaryotic gene expression cassette, which comprises a eukaryotic gene transcriptional promoter sequence, a multiple cloning site and a transcriptional terminator sequence, wherein a desired gene is inserted into said multiple cloning site;
 - (c) a lactic acid bacterial plasmid sequence, which comprises a plus origin of replication, and a nucleic acid sequence encoding a Rep A protein which is involved in replication of the lactic acid bacterial plasmid; and
 - (d) a marker gene that is not an antibiotic resistance gene and is operably linked to a promoter sequence.
2. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein said eukaryotic gene transcriptional promoter is a cytomegalovirus (CMV) promoter.
3. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein said lactic acid bacterial plasmid sequence is a plasmid of 2.1 kb in size isolated from Lactobacillus plantarum.
4. (Previously presented) The Lac shuttle vector as claimed in claim 3, wherein the protein which is involved in the lactic acid bacterial plasmid replication is a Rep A protein consisting essentially of 317 amino acids.

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5. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein said marker gene is a β -galactosidase gene.

6. (Previously presented) The Lac shuttle vector as claimed in claim 5, wherein the promoter of said β -galactosidase gene is an erythromycin resistance gene promoter.

7. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the Lac Shuttle vector comprises the nucleotide sequence set forth in SEQ ID NO:1 or a complementary nucleotide sequence thereto, or a degenerate variant thereof that contains degenerative protein-coding sequences.

8. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the Lac Shuttle vector comprises the nucleotide sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereto, or a degenerate variant thereof that contains degenerative protein-coding sequences.

9. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the Lac shuttle vector is selected from the group consisting of:

(a) pCLP7 having the configuration of restriction sites in FIG. 4, American Type Culture Collection Accession No. PTA-2661; and

(b) pCLP8 having the configuration of restriction sites in FIG. 4, American Type Culture Collection Accession No. PTA-2663.

10. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the vector is for transforming a host cell, the host cell being a Gram-positive bacterium, and the endogenous β -galactosidase gene of the host cell being non-functional.

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11. (Previously presented) The Lac shuttle vector as claimed in claim 10, wherein the host cell is the Lac- mutant of *Lactobacillus casei*, subsp. *casei*, which is designated Ana-1, American Type Culture Collection Accession No. PTA-2662.

12. (Previously presented) A kit for expression of a gene, comprising:
(a) the Lac shuttle vector as claimed in claim 1;
(b) a host cell in which the endogenous β -galactosidase gene thereof is non-functional; and
(c) a eukaryotic cell.

13. (Previously presented) A DNA immunogenic composition comprising a Lac shuttle vector that contains:
(a) a region which regulates a plasmid copy number, wherein said region comprises an *E. coli* replication origin sequence;
(b) a eukaryotic gene expression cassette, which comprises a eukaryotic gene transcriptional promoter sequence, a multiple cloning site and a transcriptional terminator sequence, wherein an antigenic gene is inserted into said multiple cloning site;
(c) a lactic acid bacterial plasmid sequence, which comprises a plus origin of replication, and a nucleic acid sequence encoding a Rep A protein which is involved in replication of the lactic acid bacterial plasmid; and
(d) a marker gene that is not an antibiotic resistance gene and is operably linked to a promoter sequence.

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14. (Previously presented) A method for selection of a host cell containing a vector, comprising:
- (i) introducing into said host cell the Lac shuttle vector as claimed in claim 1, wherein the endogenous β -galactosidase gene of said host cell is non-functional; and
 - (ii) culturing said host cell transformed in step (i) under conditions in which lactose is the only carbon source, thereby selecting a host cell comprising the Lac shuttle vector of claim 1.
15. (Cancelled)
16. (Cancelled)